Lab 4-Propyl Gallate

G25

Investigations on the Toxic and Teratogenic Effects of GRAS Substances on the Developing Chick Embryo.[1]

Propyl Gallate

Edward C. Naber
Department of Poultry Science
The Ohio State University
674 West Lane Avenue
Columbus, Ohio 43210

[1]Report of investigations conducted under Contract No. 72-343 with the Food and Drug Administration, PHS, DHEW.

General Protocol:

Ten test substances were supplied by the Food and Drug Administration for testing in the chick embryo. Details on the nature and source of these substances is shown in Table i. All substances were stored at room temperature in the dark until they were used, except that the propyl gallate and phosphated mono- and di-glycerides were kept under refrigeration. Most of the substances were dissolved in a suitable solvent or suspended in a suitable liquid for injection into fertile eggs. In one instance the substance was injected directly without a solvent or carrier. Specific information about solvents, solubility of the substances and problems peculiar to individual substances will be given under specific protocol for each substance tested.

Fertile eggs used in these investigations were from a specific pathogen free flock of Dekalb 161 egg production type chickens fed a breeder ration free of antibiotics or other drugs. Eggs were stored at 55° F and a relative humidity of 80 percent for 0 to 5 days prior to use. Eggs were allowed to reach room temperature, placed on plastic flats and subjected to ultraviolet irradiation for 30 minutes. The top of each egg was cleansed by a cotton swab saturated with 70 percent ethanol, a small hole was drilled over the air cell through the shell and the test substance was injected with the aid of a 0.25 ml. tuberculin syringe fitted with a suitable needle. All equipment and glassware used to handle the test substances or their solutions or suspensions were sterilized by auto claving and every attempt was made to avoid microbiological contamination of the eggs. Following injection the hole in each egg was sealed by a drop of flexible collodion and the eggs were set in or returned to the incubators. Jamesway Model 252 Incubator-Hatchers were used and maintained at 100° F dry bulb temperature and 86° F wet bulb temperature during the first 18 days of incubation. Eggs were turned automatically each 4 hours. Eggs were candled periodically to remove dead embryos and all embryos were examined for stage of development and obvious defects. After 18 days of incubation viable embryos were transferred to hatching baskets and hatching temperature was reduced to 98.5° F dry bulb reading and humidity was increased to a 90° F wet bulb reading. Upon hatching (22nd day) chicks were examined for abnormalities and samples were cleared and alizarin stained to examine them for skeletal defects. Other embryos (50 for each substance studied) were sacrificed and samples of liver, muscle, bursa, brain, eye, spleen, heart, pancreas, lung and kidney were taken and fixed in formalin. Later tissues were embeded in paraffin, cut, stained and mounted for histopathological examination. Each sample was done in duplicate and hence a total of 10,000 tissues were examined for lesions.

Preliminary range finding experiments were conducted to find the doses of the test substances that could be used in constructing dose response curves for toxicity as measured by embryonic mortality. In two cases, the test substance was non-toxic in the largest dose that could be accommodated by injection. Specific dose response experiments using 100 or more eggs per dose and 5 or more doses of the test substance were conducted at a minimum of 3 time intervals to obtain the toxicity data reported. Solvent or sham injected controls and untreated control groups of eggs were used with each experiment. In some cases, extra trials were conducted to provide embryos for examination at critical doses of the test substances in order to further evaluate teratogenic response and obtain additional data on the nature of embryonic defects.

Data obtained from the experiments (except that from the range finding studies) was transferred to data sheets provided (FDH form 2572, 2572a and 2572b) and submitted to FDA for statistical analysis. Nine types of data summaries including 2 statistical treatments of the data were provided by FDA on the data submitted. The results presented and interpretations made are largely based on these data summaries.

Table i

FDA Project Test Substances

Test Substance and Identification		Compound No.
1. Lactose, Edible Formost Dairies, Inc. Appleton, Wisc.		000063423
2. Propyl Gallate Lot 337		000121799
3. Sodium Ascorbate, U.S.P. FCC Lot No. 965102 Hoffmann-LaRoche Inc., Nutley, N. J. FDA 3167 73(C)		000134032
4. Sodium Erythorbate F.C.C. Lot No. 834072 FDA 3167 73(C) Hoffmann-LaRoche, Nutley, N. J.		977052064
5. Oil Nutmeg NF, East Indian Fritzsche Dodge & Olcott, Inc. 71-28 New York, N. Y.		MX 8008455
6. Zinc Sulfate - Rayon Lot # 2132R1 Virginia Chemicals, Inc. Portsmouth, Va.	Anhyd. Monohyd.	007733020 007446197
7. Stannous Chloride, AR $2H_2O$ Mallinckrodt Chemical Works St. Louis, Mo.		007772998
8. Talc USP #141, Whittaker, Clark and Daniels, Inc.		010101390
9. Carob Bean Gum FDA 71-14		PM 9000402
10. Phosphated Mono- and Di-Glycerides Lot No. 126 Witco Chemical Organics Division New York, N. Y. EMCOL D70-30C		977051323

General Discussion and Comparisons:

A comparison of the relative toxicity of the ten compounds tested is shown in Table ii. When toxicity is evaluated by the air cell route of injection at 96 hrs. of incubation, which was the most sensitive for most of the substances tested, it may be seen that the test substances can be divided into 3 categories of toxicity. Substances highly toxic are zinc sulfate, propyl gallate and carob bean gum. Moderate toxicity was encountered with sodium ascorbate, sodium erythorbate, oil of nutmeg and stannous chloride. Those substances of low toxicity were lactose, talc and phosphated mono- and di-glyceride.

Most of the substances tested produced general embryo toxic response as ascites and/or edema except for lactose and talc at the doses tested. Some specific structural defects were noted and seemed to be related to certain substances as shown in Table ii.

Table ii

Comparison of Ten Substances Tested
for Toxicity and Teratology

Substance Tested	LC_{50} via air cell at 96 hrs.	Specific Abnormalities Noted
Lactose	very large	none
Propyl Gallate	13 mgs./kg.	Ascites, edema, celosomia.
Sodium Ascorbate	100 mgs./kg.	Ascites, edema, celosomia, liver histopathology, head defects.
Sodium Erythorbate	84 mgs./kg.	Ascites, liver histopathology.
Oil of Nutmeg	240 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Zinc Sulfate	4 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Stannous Chloride	120 mgs./kg.	Ascites, edema, celosomia.
Talc	>200 mgs./kg.	none
Carob Bean Gum	23 mgs./kg.	Anophthalmia, phocomelia, micromelia, torticollis, celosomia.
Phosphated Mono- and Di-Glycerides	>3000 mgs./kg.	Ascites, anophthalmia, brachygnathia.

II. PROPYL GALLATE

Specific Protocol:

Propyl gallate is highly soluble in pure ethanol but poorly soluble in water. Hence ethanol solutions of propyl gallate were made and diluted with water to a final concentration of 20 percent ethanol. This permitted sufficient solubility of the test substance without encountering an increase in mortality in the solvent injected controls. Five doses of propyl gallate were tested at both 0 and 96 hrs. of incubation and via both air cell and yolk routes of administration.

Results:

The data for propyl gallate is presented in Tables 5-8. Doses of 1.0 mg./egg or more significantly increase percent mortality and the regression of dose on mortality was highly significant at 0 hr. in the air cell. The increase in mortality was larger and a highly significant increase was observed at a dose of 0.5 mg./egg when the compound was given at 96 hrs. in the air cell. Again the regression of dose on mortality was highly significant. Yolk administration of propyl gallate at 0 hr. failed to increase percent mortality significantly but it should be noted that solvent control mortality is much greater when this route of administration is used at 0 hr. When given at 96 hrs. in the yolk, propyl gallate again significantly increase percent mortality at 1.0 mg./egg or more and the regression of dose on mortality was highly significant.

The percent abnormal chicks hatched was significantly increased by the higher doses when given via the air cell at 0 hr. Due to very high mortality at 96 hrs. a significant decrease in percent abnormal chicks was noted when propyl gallate was given via the air cell. The latter effect was also observed at 96 hrs. with the highest level of yolk injection. No significant effect of propyl gallate was observed on percent abnormal chicks when it was given at 0 hr. via the yolk.

Percent of H-S-V-L abnormalities was significantly greater at the 3 higher levels of propyl gallate when given at 0 hr. via the air cell and at the 1.0 mg./egg dose when given at 96 hrs. via the air cell. No significant differences were noted for yolk administration at either time interval.

The most frequent embryonic defects noted were ascites in the abdomen and general edema. Celosomia was also regularly observed in this study. There appeared to be some relationship of celosomia to propyl gallate level at 0 hr. when given via the air cell. Ascites and general edema appeared to increase with dose under all conditions of compound administration except for the 96 hrs. air cell administration where mortality was very high.

Discussion:

Propyl gallate clearly produces an embryo toxic response that is closely related to the dose administered. This is evidenced by a high level of embryonic mortality and an increase in the number of abnormal chicks hatched when mortality was between 30 and 60 percent via air cell treatment. The increase in celosomia accounts for the significant rise in H-S-V-L abnormalities observed when the substance was given via the air cell. The LC_{50} at 96 hrs. via the air cell was about 13 mgs./kg.

PROPYL GALLATE (MG/KG) IN 20% ETHANOL/H2O ONE GD MORE ABNORMALITIES
PROPYL GALLATE (MG/KG) IN 20% ETHANOL/H2O MORTALITY PCT
PER CENT
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00

Lab 4

⊖ LAB 4 PROPYL GALLATE (MG/KG) IN 20% ETHANOL/Y/095 ONE OR MORE ABNORMALITIES
✱ LAB 4 PROPYL GALLATE (MG/KG) IN 20% ETHANOL/Y/095 MORTALITY PCT

Lab 4

PROPYL GALLATE (MG/KG) IN 20% ETHANOL/H/22G ONE OR MORE ABNORMALITIES
PROPYL GALLATE (MG/KG) IN 20% ETHANOL/G/22G MORTALITY PCT
PER CENT
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00

Lab 4

LAB 4 PROPYL GALLATE (MG/KG) IN 20% ETHANOL/Y/000 ONE OR MORE ABNORMALITIES
LAB 4 PROPYL GALLATE (MG/KG) IN 20% ETHANOL/Y/000 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
100.00
88.00
75.00
63.00
50.00
38.00
25.00
13.00
00.00

Lab 4

Table 5

DATA SUMMARY

Propyl Gallate in 20% Ethanol
via Air Cell at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	465	11.18	10.32	3.01
Solvent	None	114	12.28	10.52	1.75
10.0	0.5	98	19.38	19.38	5.10
20.0	1.0	118	30.50[1]	23.72[2a]	5.08
40.0	2.0	114	52.63[1]	22.80[2a]	12.28[3]
60.0	3.0	118	65.25[1]	28.81[2]	11.01[3a]
80.0	4.0	120	80.00[1]	31.66[2]	16.66[3]

[1] Difference from control group is highly significant

[2] Difference from control group response is highly significant

[2a] Difference from control group response is significant

[3] Same as 2

[3a] Same as 2a

[4] Regression of dose on mortality is highly significant

LC_{30} = 25 mgs./kg.
LC_{50} = 43 mgs./kg.
LC_{70} = 73 mgs./kg.
LC_{90} = 159 mgs./kg.

[5] NS - F (Cal) $<$ F (.05)

Table 6

DATA SUMMARY

Propyl Gallate in 20% Ethanol
via Air Cell at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	465	11.18	10.32	3.01
Solvent	None	120	7.50	12.50	1.66
10.0	0.5	129	40.83[1]	20.83	5.83
20.0	1.0	118	71.18[1]	21.18	8.47[3]
40.0	2.0	116	97.41[1]	0.86[2]	0.86
60.0	3.0	118	95.76[1]	2.54[2a]	0
80.0	4.0	118	100.00[1]	0[2]	0

[1] Difference from control group is highly significant

[2a] Difference from control group response is significant

[2] Difference from control group response is highly significant

[3] Same as 2a

[4] Regression of dose on mortality is highly significant

LC_{30} = 9.0 mg/kg
LC_{50} = 13.3 mg/kg
LC_{70} = 19.6 mg/kg
LC_{90} = 34.1 mg/kg

[5] Slope is negative

Table 7

DATA SUMMARY

Propyl Gallate in 20% Ethanol
via Yolk at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	465	11.81	10.32	3.01
Solvent	None	68	32.35	13.23	4.41
10.0	0.5	112	39.28	11.60	6.25
20.0	1.0	92	46.73	8.69	3.26
40.0	2.0	90	48.88	17.77[2]	5.55
60.0	3.0	100	48.00	16.00	4.00
80.0	4.0	106	57.54[1]	15.09	6.60[3]

[1] NS
[2] NS
[3] NS
[4] F (Cal) $\leq$ F (.05)
[5] F (Cal) $\leq$ F (.05)

Table 8

DATA SUMMARY

Propyl Gallate in 20% Ethanol
via Yolk at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	465	11.18	10.32	3.01
Solvent	None	118	16.10	15.25	4.23
10.0	0.5	116	24.13	22.41	2.58
20.0	1.0	113	30.08[1a]	17.69	2.65
40.0	2.0	120	74.16[1]	9.16	1.66
60.0	3.0	109	70.64[1]	17.43	2.75[3]
80.0	4.0	120	93.33[1]	5.83[2]	1.66

[1] Difference from control group is highly significant

[1a] Difference from control group is significant

[2] Difference from control group is significant

[3] NS

[4] Regression of dose on mortality is highly significant
LC_{30} = 22.5 mg/kg
LC_{50} = 33.9 mg/kg
LC_{70} = 51.2 mg/kg
LC_{90} = 92.6 mg/kg

[5] Slope is negative